Probing Antibody-Target Interactions in Vivo

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UNC at Chapel Hill

Part I: Why do we care about “antibody-target interaction” in vivo?

Part II: How do we quantify “antibody-target interaction” in vivo?

Part I: Why do we care about “antibody-target interaction” in vivo?

Part II: How do we quantify “antibody-target interaction” in vivo?

Antibody Products Grow Rapidly

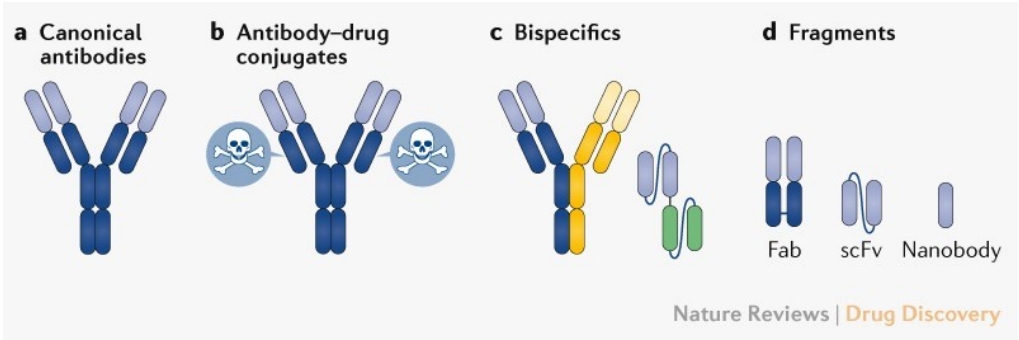


Table 1 | **Top targets for first 100 mAbs**

Target	mAb count
PD1/PDL1	7
CD20	6
TNF	4
HER2	4
CGRP/CGRPR	4
VEGF/VEGFR	4
IL-6/IL-6R	4
IL-23 p19	3
EGFR	3
CD19	3

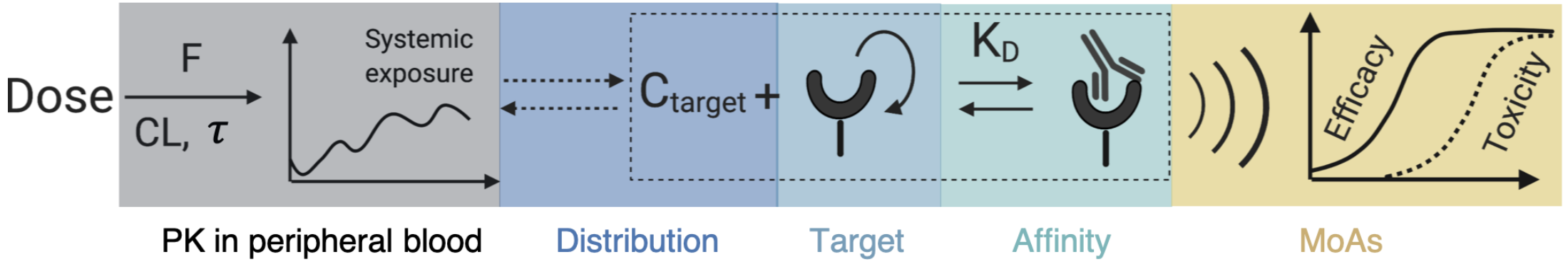
Table 2 | **Top investigational mAb targets**

Target	Investigational agent count[a]
PD1/PDL1	80[b]
CD3	71
HER2	34
CTLA4	25
SARS-CoV-2	22
4-1BB	19
LAG3	19
EGFR	17
CD20	15
CD47	15

1. Poor accessibility to distal targets.
2. High resistance.

Mullard A, ***Nat Rev Drug Discov***. 2021

The Cascade of Pharmacological Action



Antibody Dose – Exposure

PopPK

(1) Target Exposure

PBPK

(2) Target Engagement

TMDD

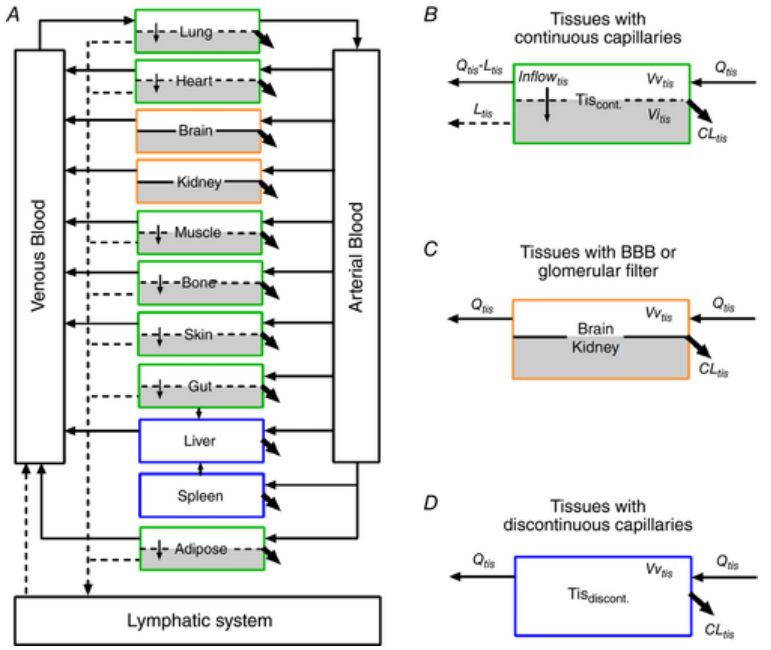
(3) MoAs

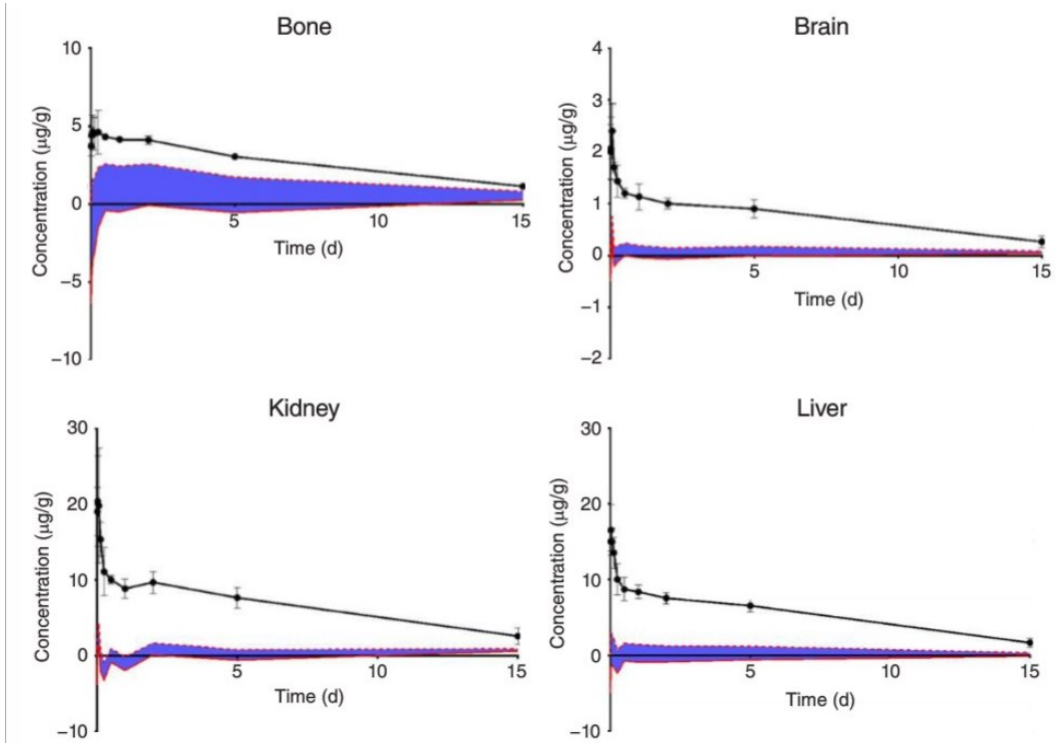
QSP

Antibody Exposure – Response (often ambiguous)

E-R analysis

Antibody Tissue Exposure is Challenging to Measure

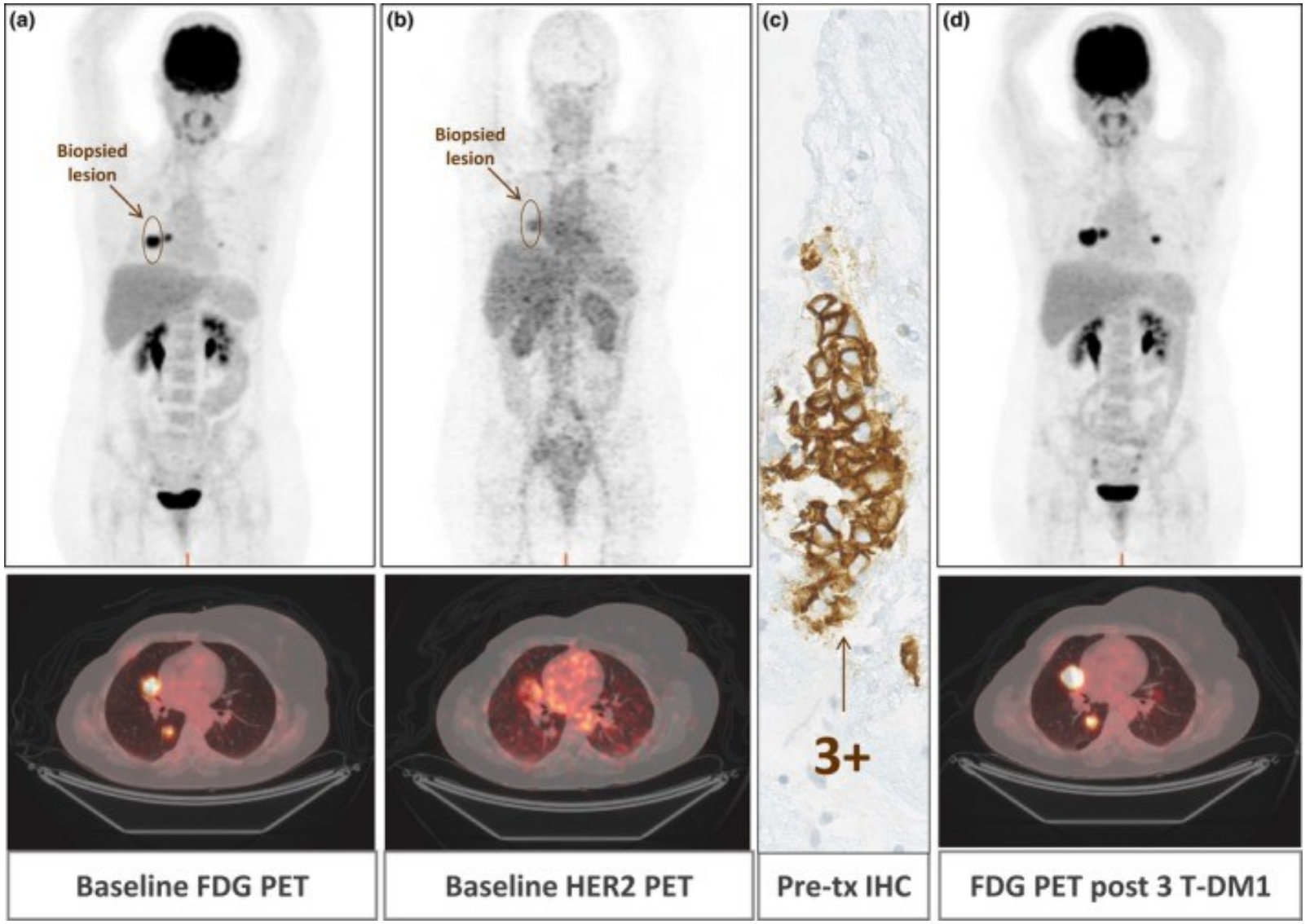




Physiologically-based Pharmacokinetic model (PBPK)

Wiig H. ***J Physiol***. 2017

Poor correlation between expression (IHC) and ^{89}Zr-trastuzumab uptake



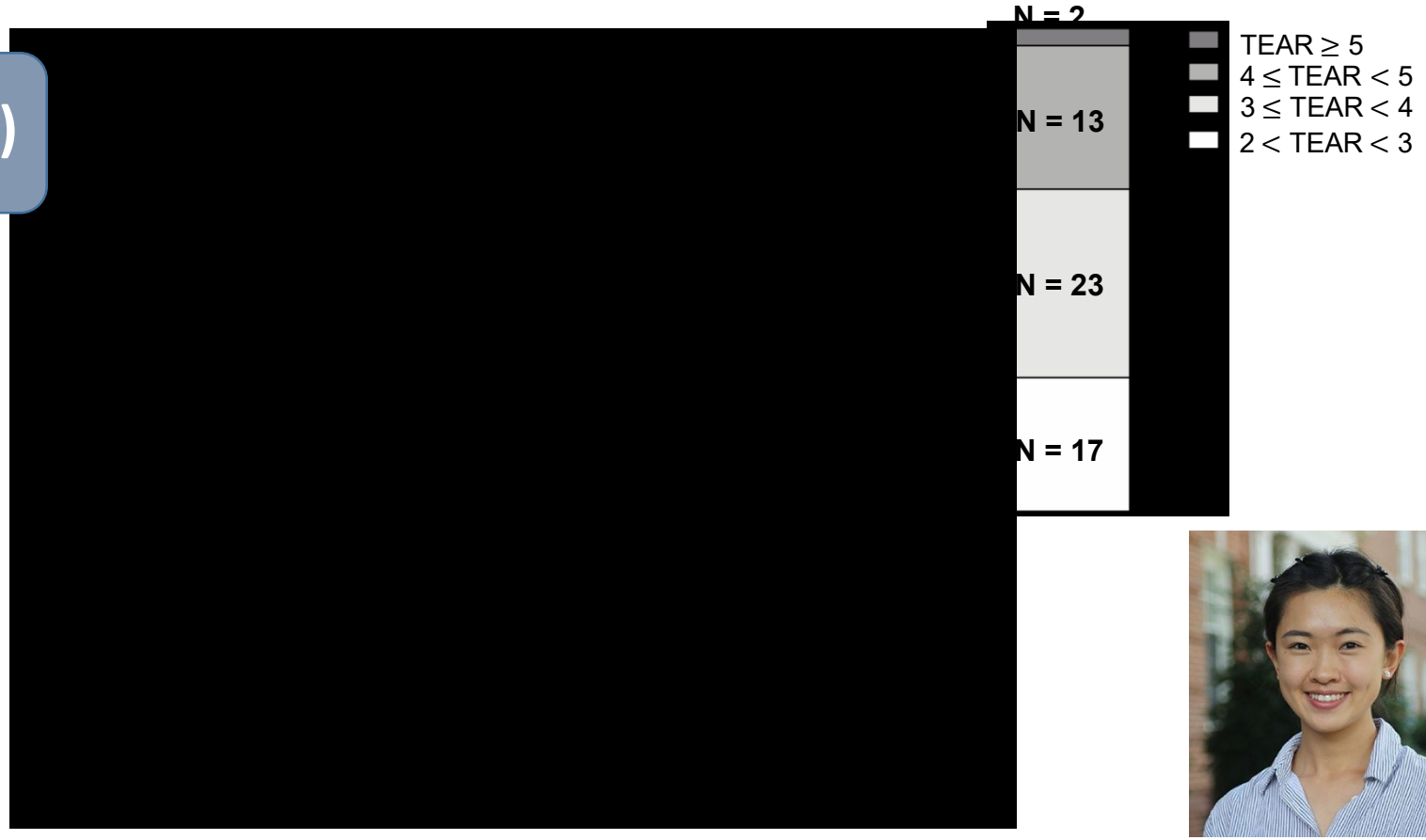
Bartelink IH, ***Clin Pharmacol Ther***. 2019

TEAR (Therapeutic Exposure Affinity Ratio)

$$TEAR = \log\left(\frac{\text{Plasma Exposure at TD}}{\text{Affinity } (K_D \text{ in vitro})}\right)$$

TEAR = 2	RO = 99%
TEAR = 3	RO = 99.9%

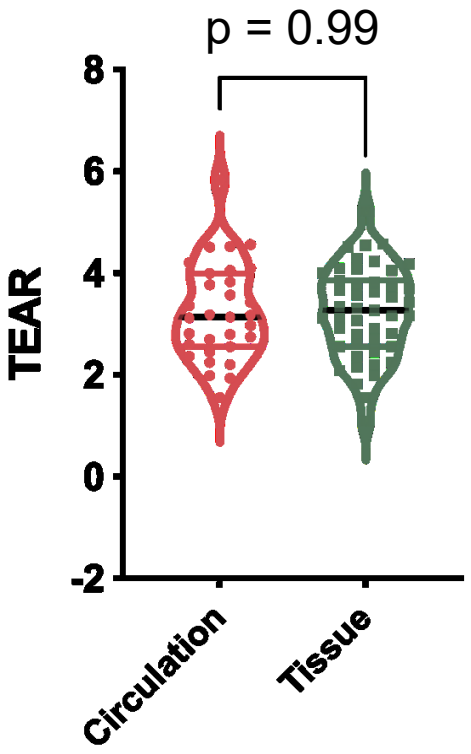
Proof-by-Contradictions

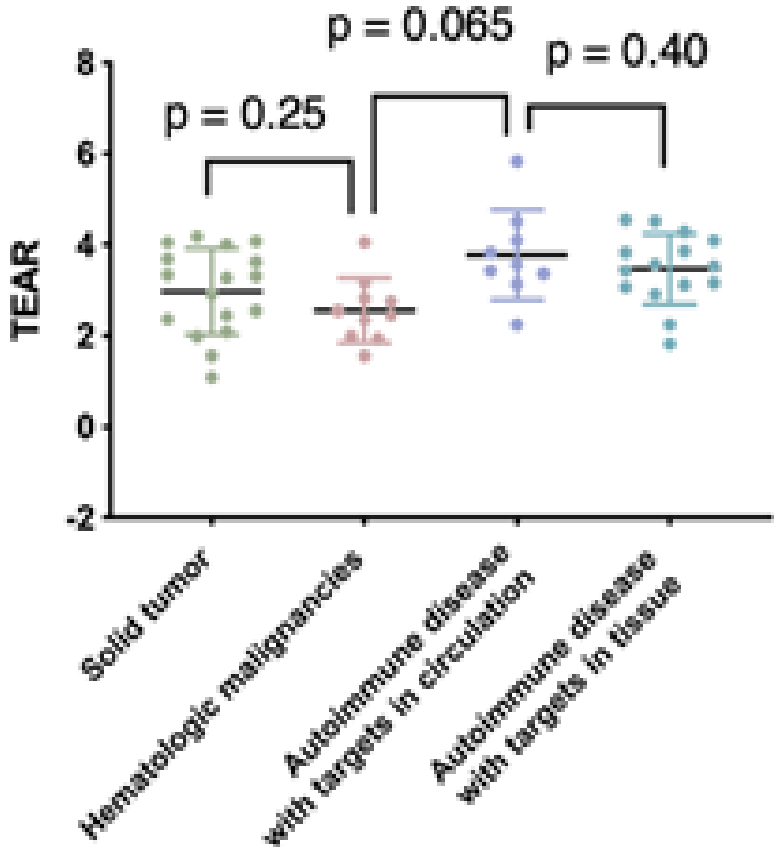


Tang Y.

Tang Y, ***Drug Discov Today***. 2021

Location or Affinity Assumptions: Which is More Biased?



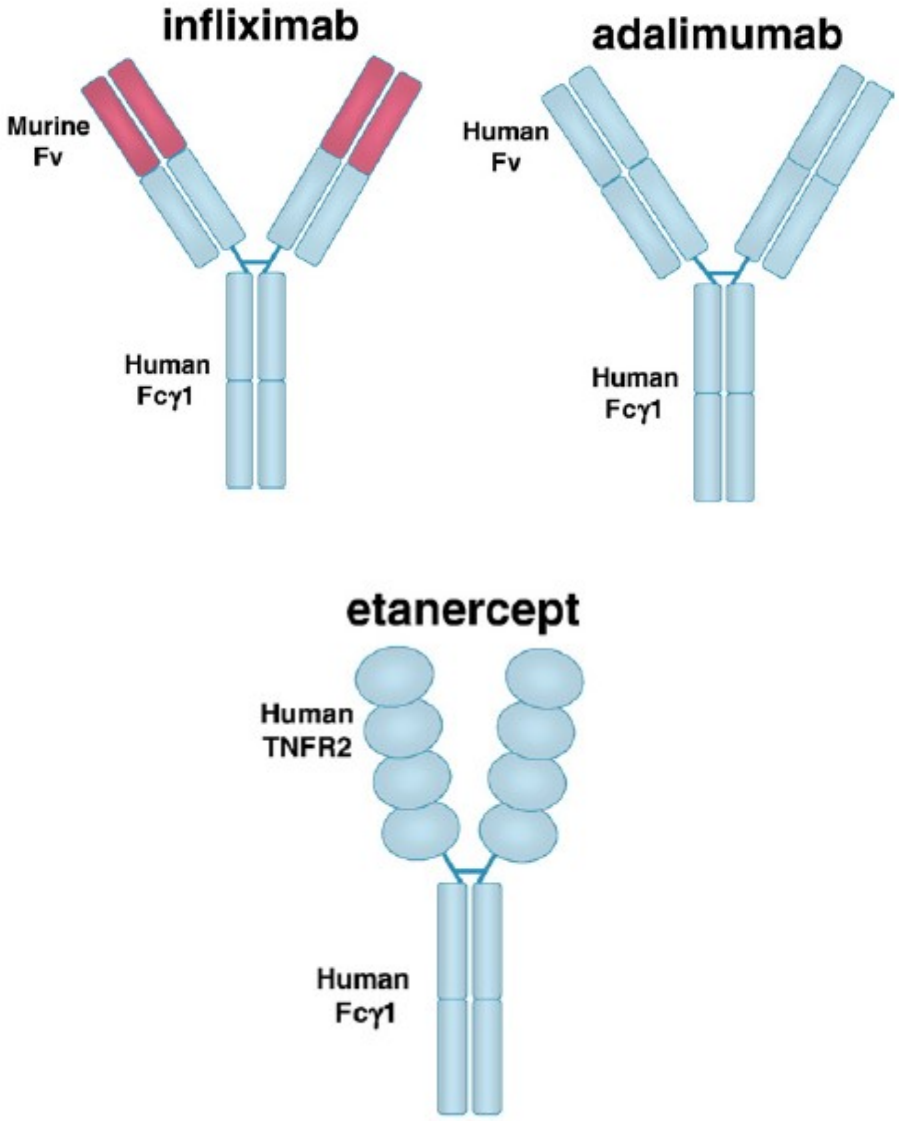


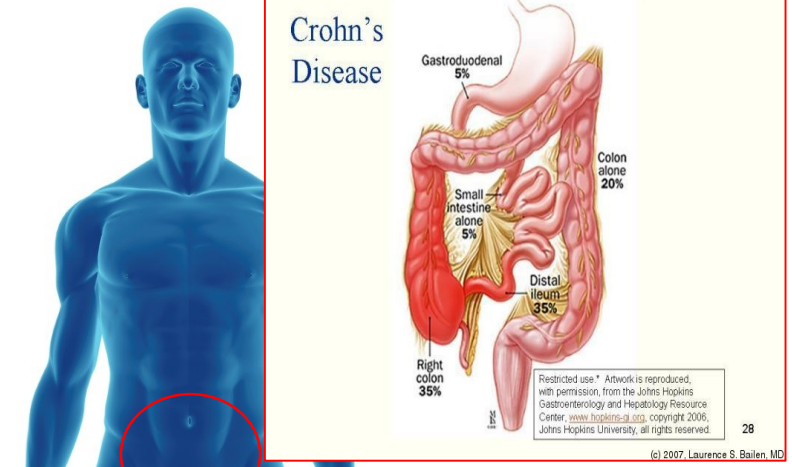
Tang Y, ***Drug Discov Today***. 2021

Antibody-Target Interaction: Close vs. Open Systems

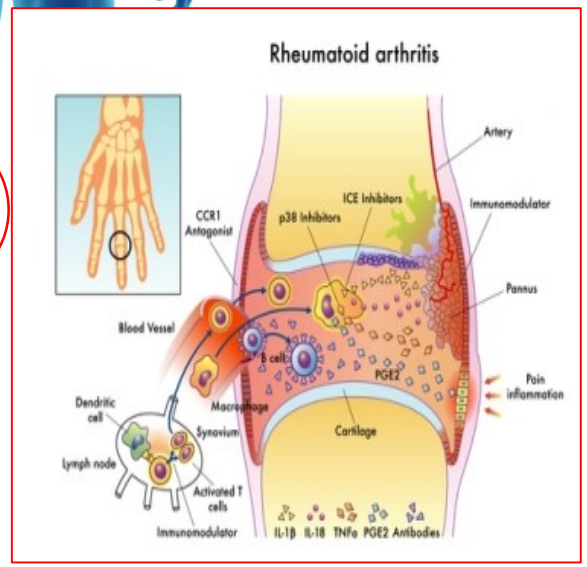
Pan AC, ***Drug Discov Today***. 2013

For instance: TNF-α a target for autoimmune diseases



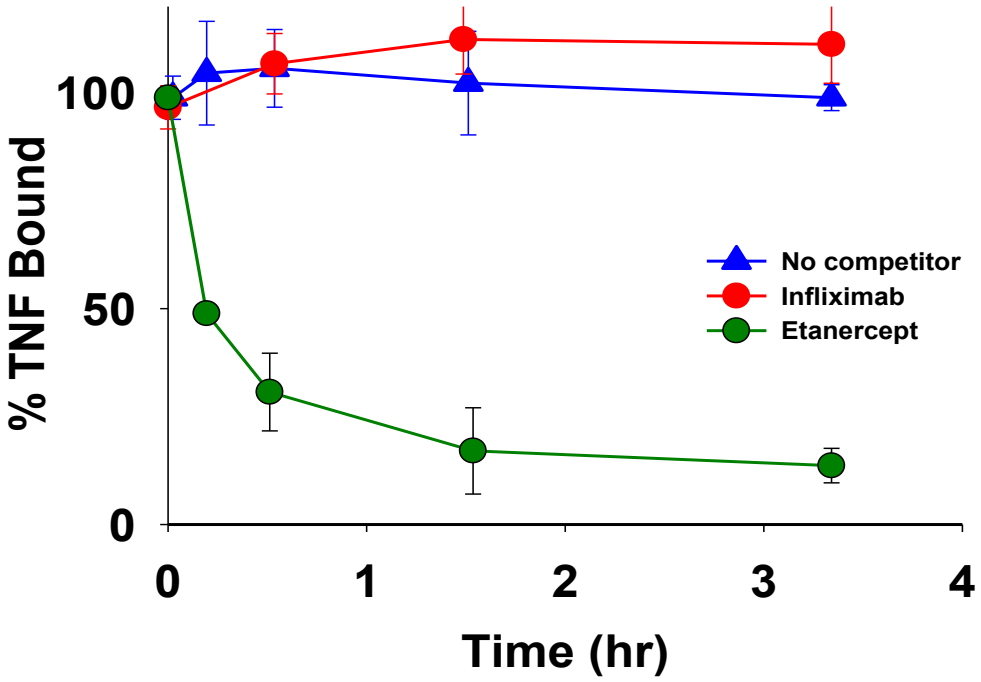


Crohn's Disease



Why are they different in clinical effect?

- **Receptor Binding Kinetics.**
- **Complex Stability**



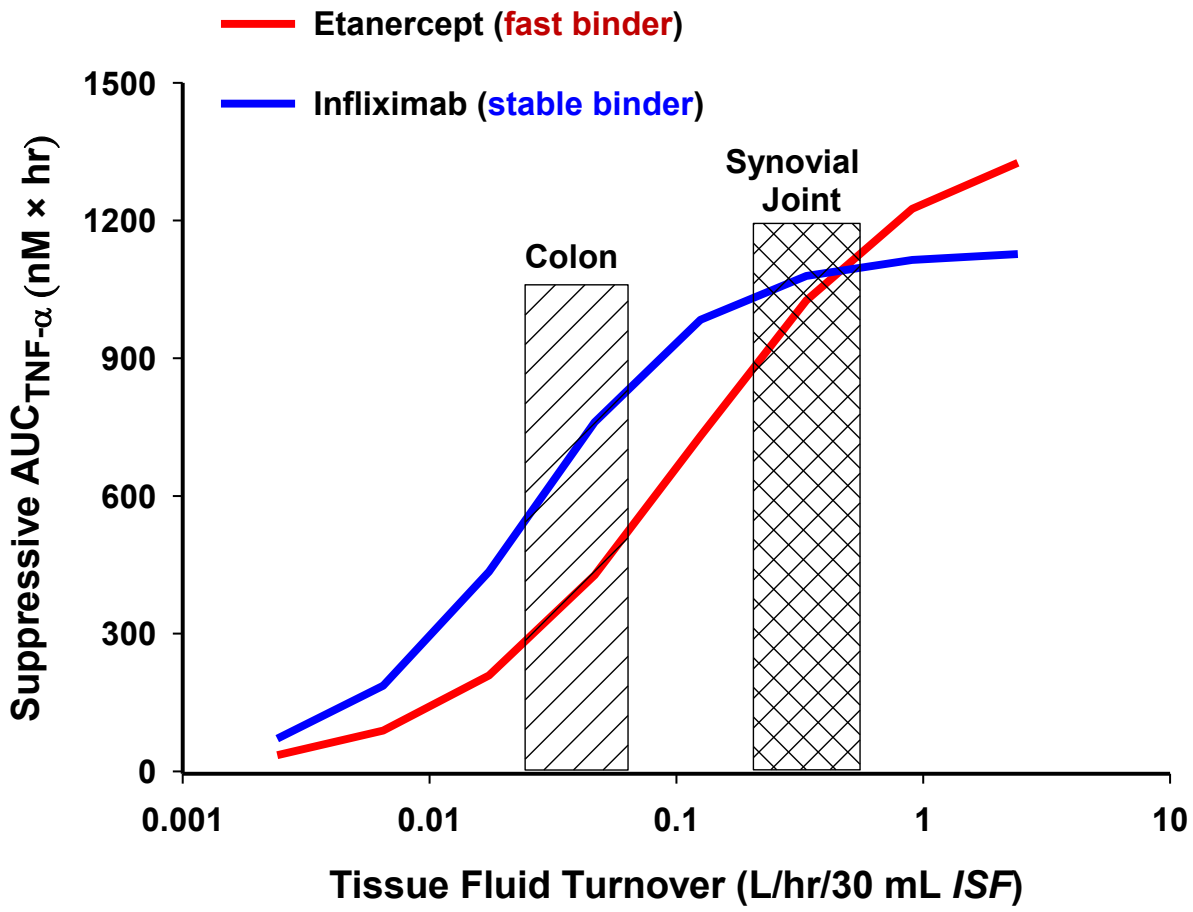
	K_{on} (10^5 $M^{-1}S^{-1}$)	K_{off} ($10^{-4}S^{-1}$)	K_D (nM)
Infliximab	0.57	1.1	1.92
Etanercept	2.6	7	2.31

Scallon B., ***J Pharmacol Exp Ther***. 2002
Santora LC, ***Anal Biochem***. 2001
Kim MS, ***J. Mol Biol*** 2007
Song MY, ***Exp. Mol Med*** 2008

The interstitial fluid turnover is different

<

Effect vs "Tissue fluid turnover"

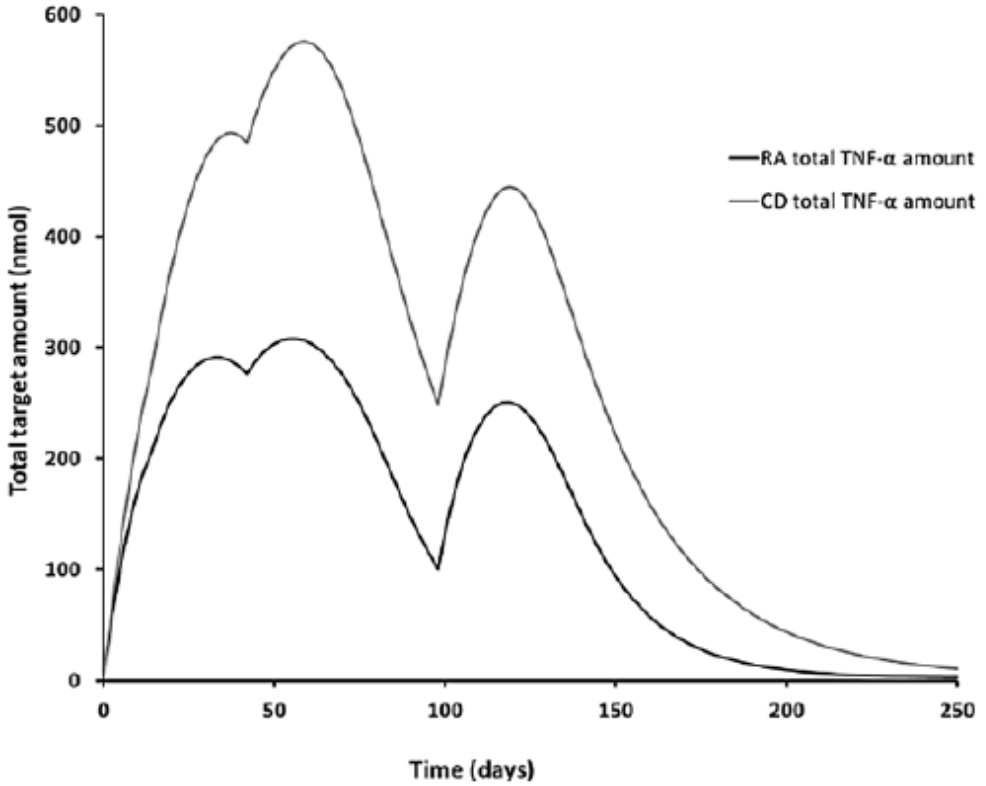


Clinical Pharmacokinetics
https://doi.org/10.1007/s40262-021-01057-3

ORIGINAL RESEARCH ARTICLE

Infliximab Treatment Does Not Lead to Full TNF-α Inhibition: A Target-Mediated Drug Disposition Model

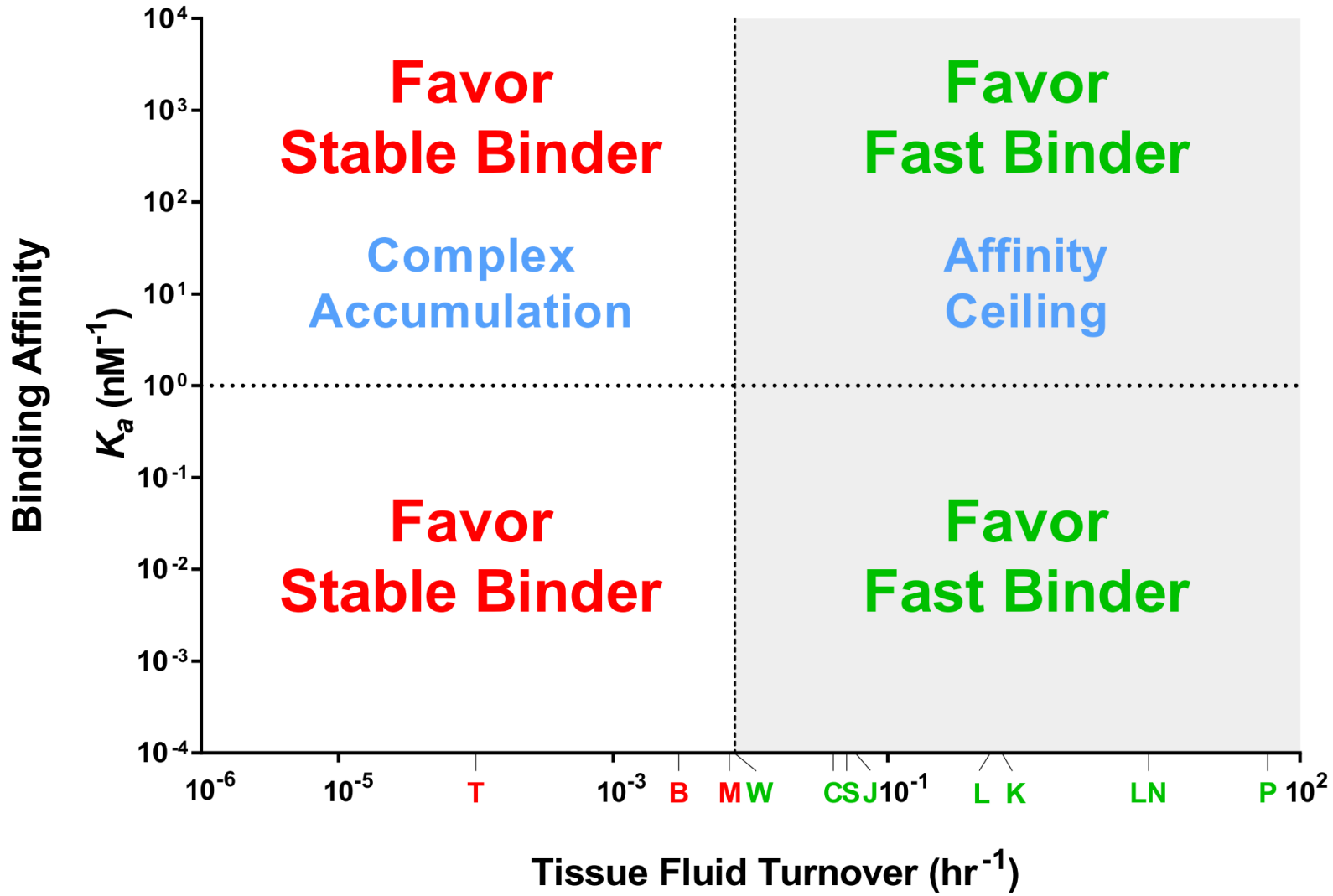
David Ternant[1,2,3,9] · Marc Pfister[1] · Olivier Le Tilly[2,3] · Denis Mulleman[4,5] · Laurence Picon[6] · Stéphanie Willot[7] · Christophe Passot[8] · Theodora Bejan-Angoulvant[2,3] · Thierry Lecomte[4,6] · Gilles Paintaud[2,3] · Gilbert Koch[1]



Complex elimination was much **slower in Crohn's disease** than in RA (k_{int} = 0.024 vs 0.061 day^{-1})

Li X, ***J Pharmacol Exp Ther***., 2019
Ternant D, ***Clin Pharmacokinet***. 2021

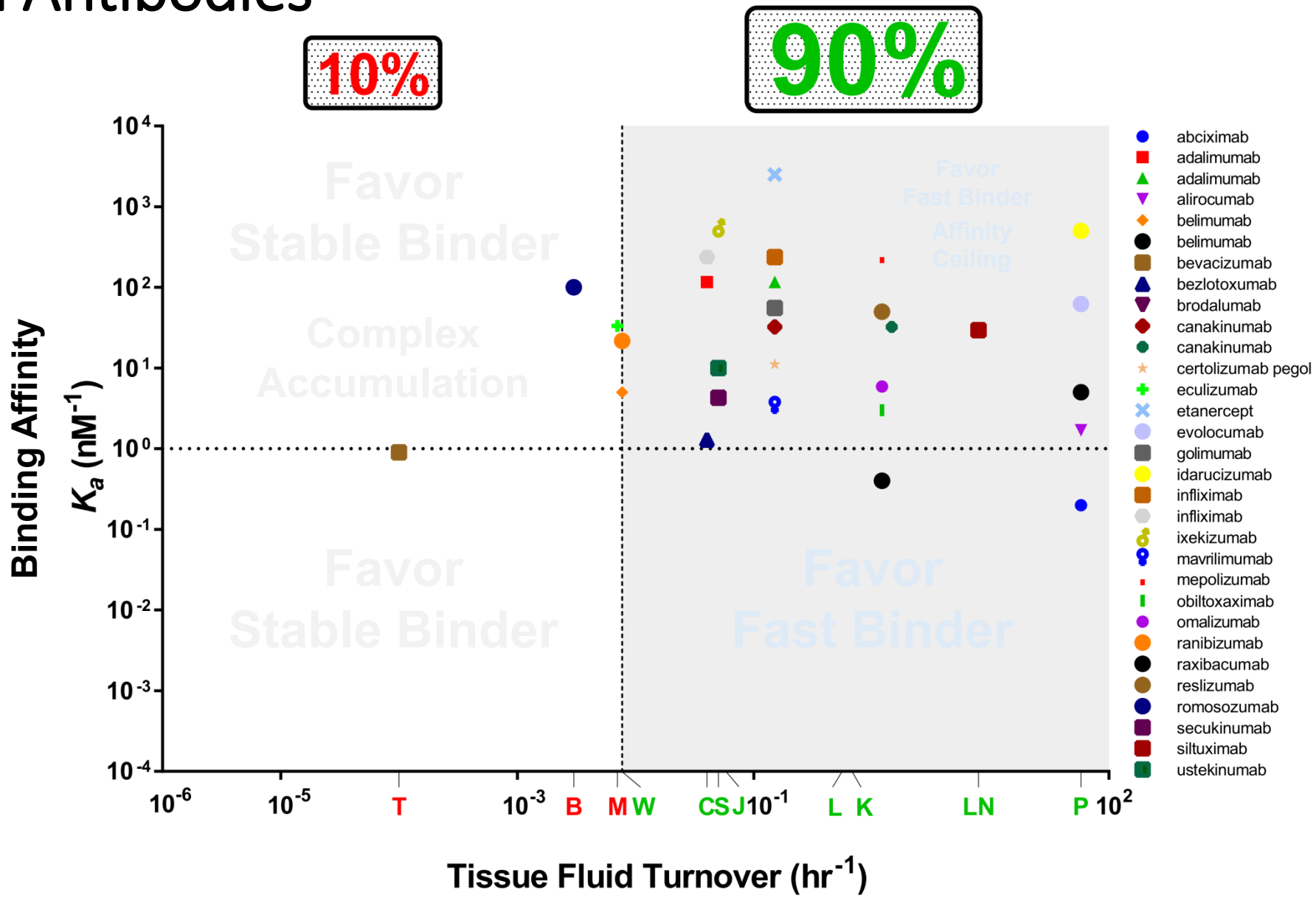
Binding Affinity and “Tissue fluid turnover”



T: Tumor, B: Bone, M: Muscle, W: Whole Body, C: Colon, S: Skin,
J: Joint (synovial fluid), L: Lung, K: Kidney, LN: Ly. Node P: Plasma

Li X, ***J Pharmacol Exp Ther.***, 2019

Licensed Antibodies



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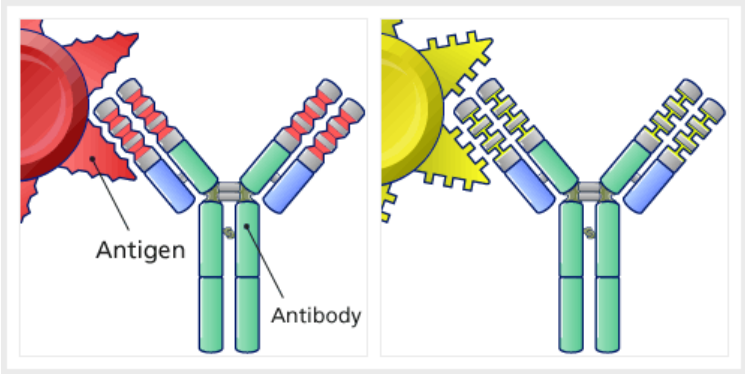
Li X, ***J Pharmacol Exp Ther.***, 2019

Summary (Part I)



1. Antibody target exposure is usually low and hard to quantify.
2. Antibody-target Interaction is context-dependent.

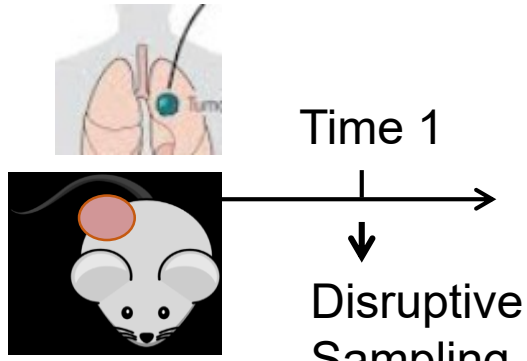
Part I: Why do we care about “antibody-target interaction” in vivo?

Part II: How do we quantify “antibody-target interaction” in vivo?

Current Technologies for Detecting RO in Solid Tumors

Disruptive Methods

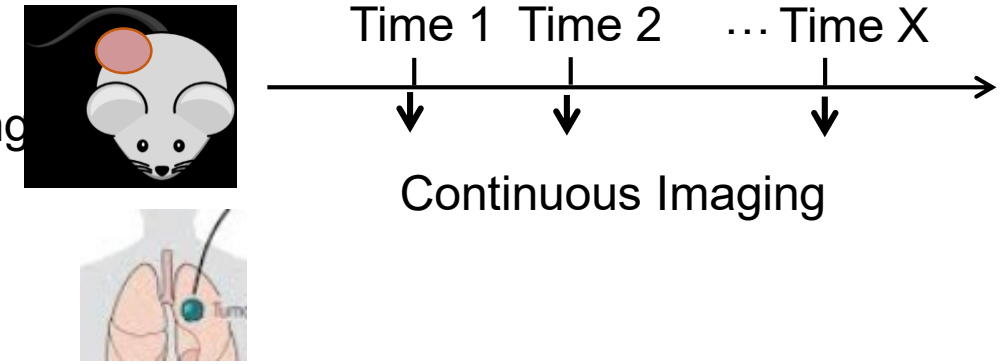
- ELISA
- LC-MS
- Immunohistochemistry
- Immunofluorescence



No spatial or temporal resolution

Noninvasive Methods

- PET
- Fluorescence imaging



Tang Y Shared Slides

Current Technologies for Detecting RO in Solid Tumors

Disruptive Methods

- ELISA
- LC-MS
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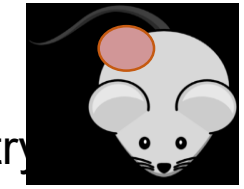
- Immunofluorescence



Time 1

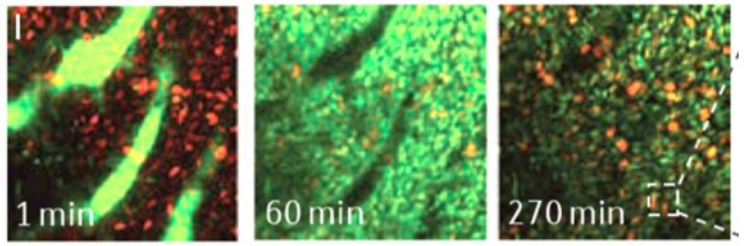
Disruptive Sampling

No spatial or temporal resolution

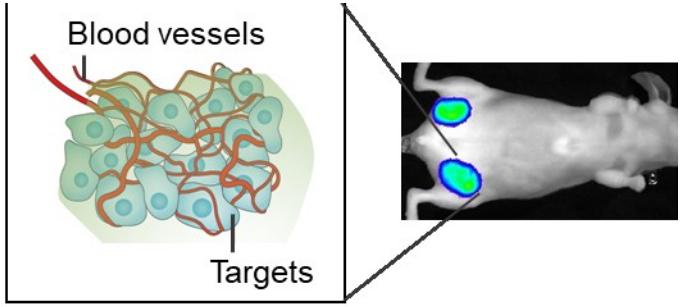
Noninvasive Methods

- PET
- Fluorescence imaging

Total Signal ≠ Bound Antibody



Microscopic Level

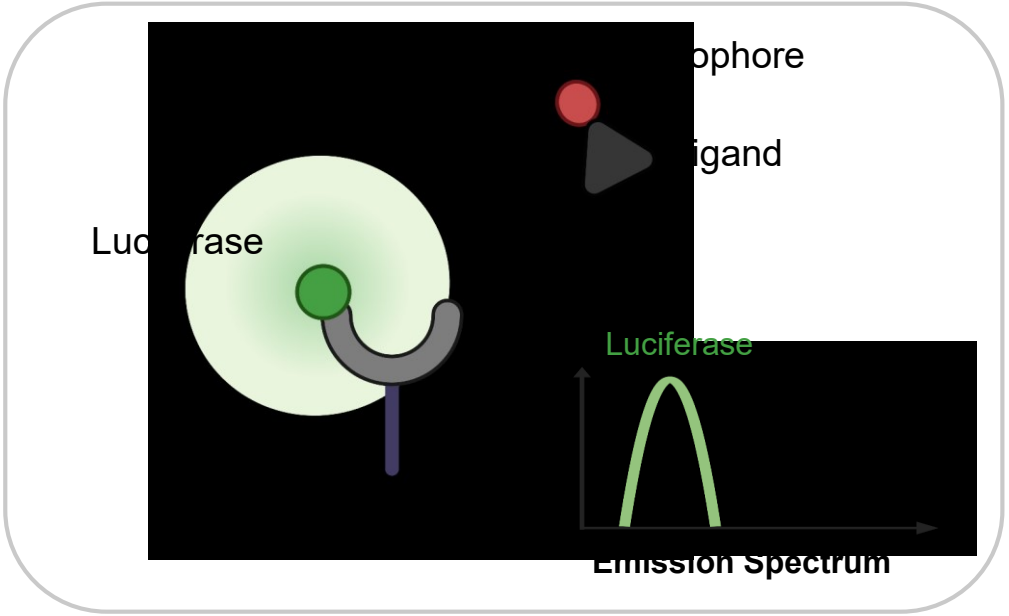


Animal Level

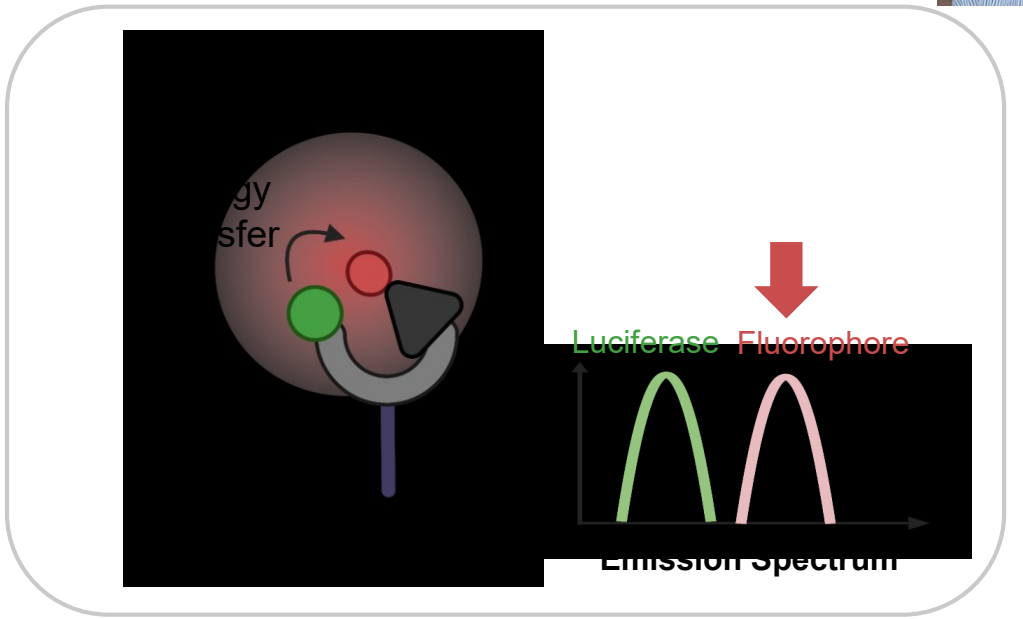
[Tang Y Shared Slides]

A BRET Approach for Detecting RO in Solid Tumors

Tang Y.



No Binding, No BRET



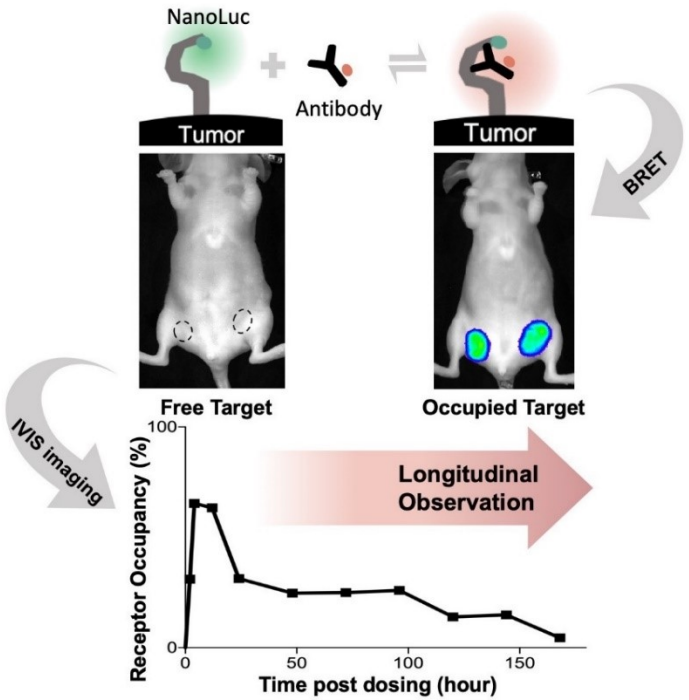
BRET exclusively reveals interactions

Promega Nanoluc plasmid

Cetuximab - EGFR

Tang Y Shared Slides

Elucidating Antibody Binding Dynamics in Living Tumors



Hypothesis:

1. Antibody-target binding dynamics in living tumors can be monitored by BRET imaging.

2. Antibody-target binding dynamics in living tumors is different with in the in vitro conditions.

3. Antibody-target binding dynamics is heterogenous in different regions of solid tumors.

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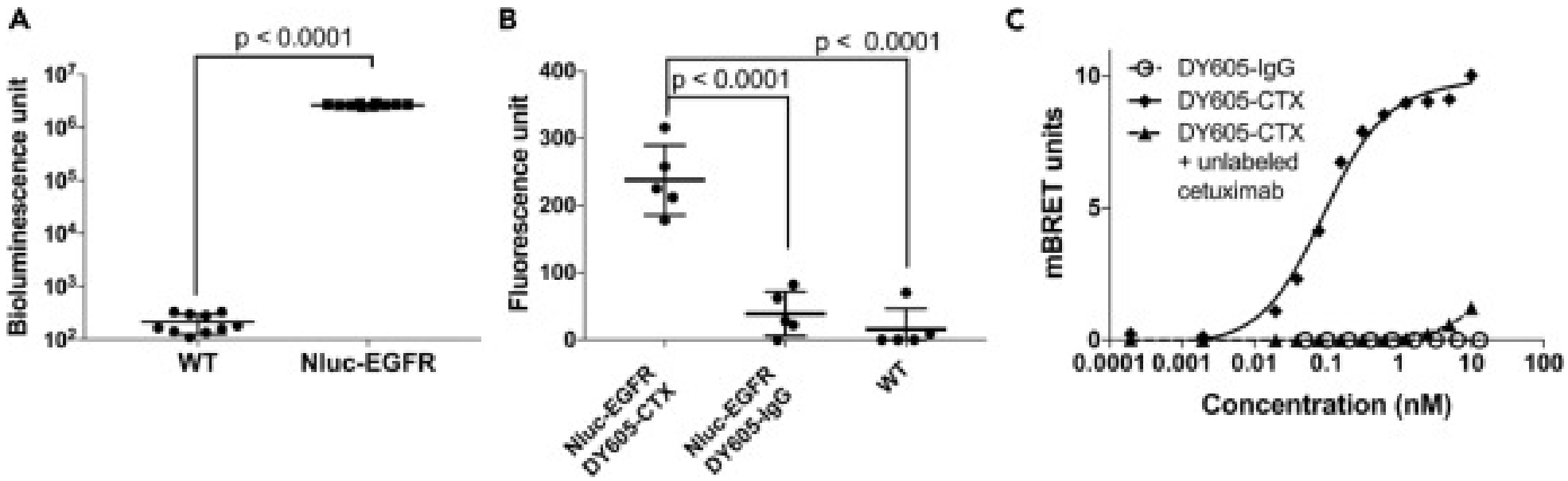
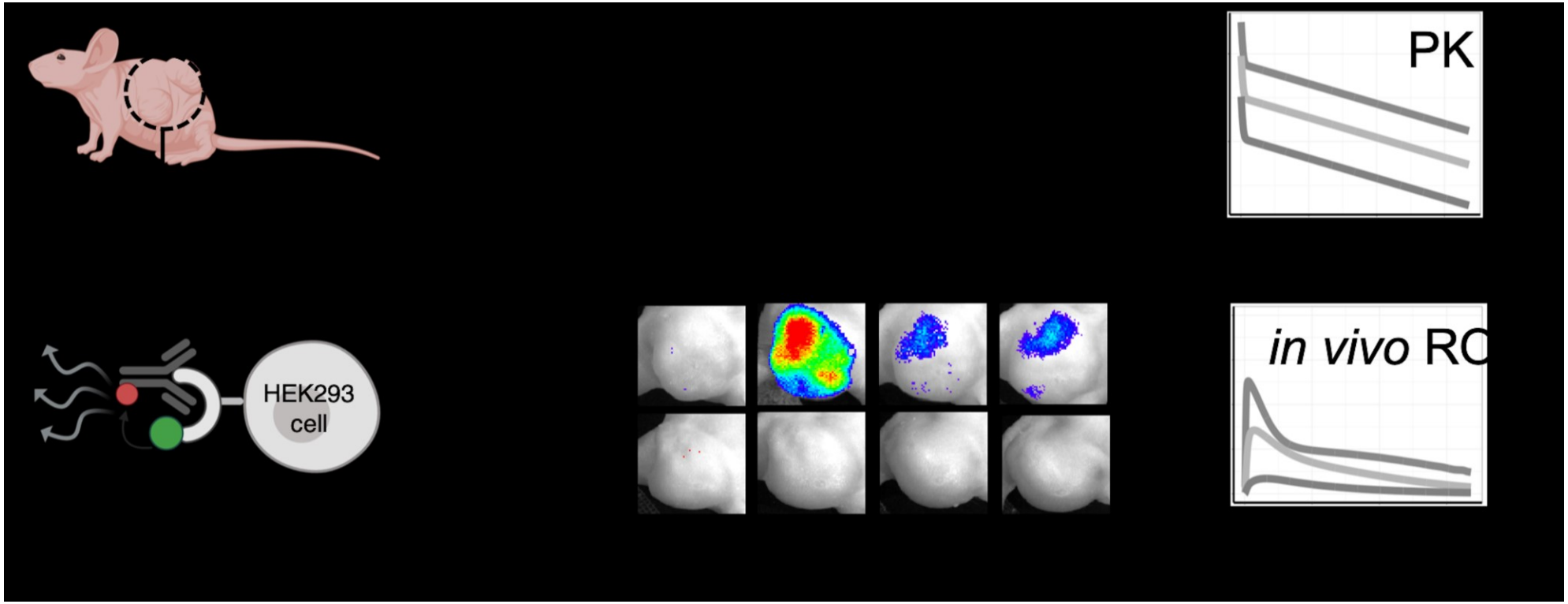
In Vitro Assay: DY605-Antibody Binds Nluc-EGFR



Tang Y Shared Slides

*Tang Y , **iScience***. 2019

Study Design



CTX: Cetuximab

Longitudinal in vivo imaging

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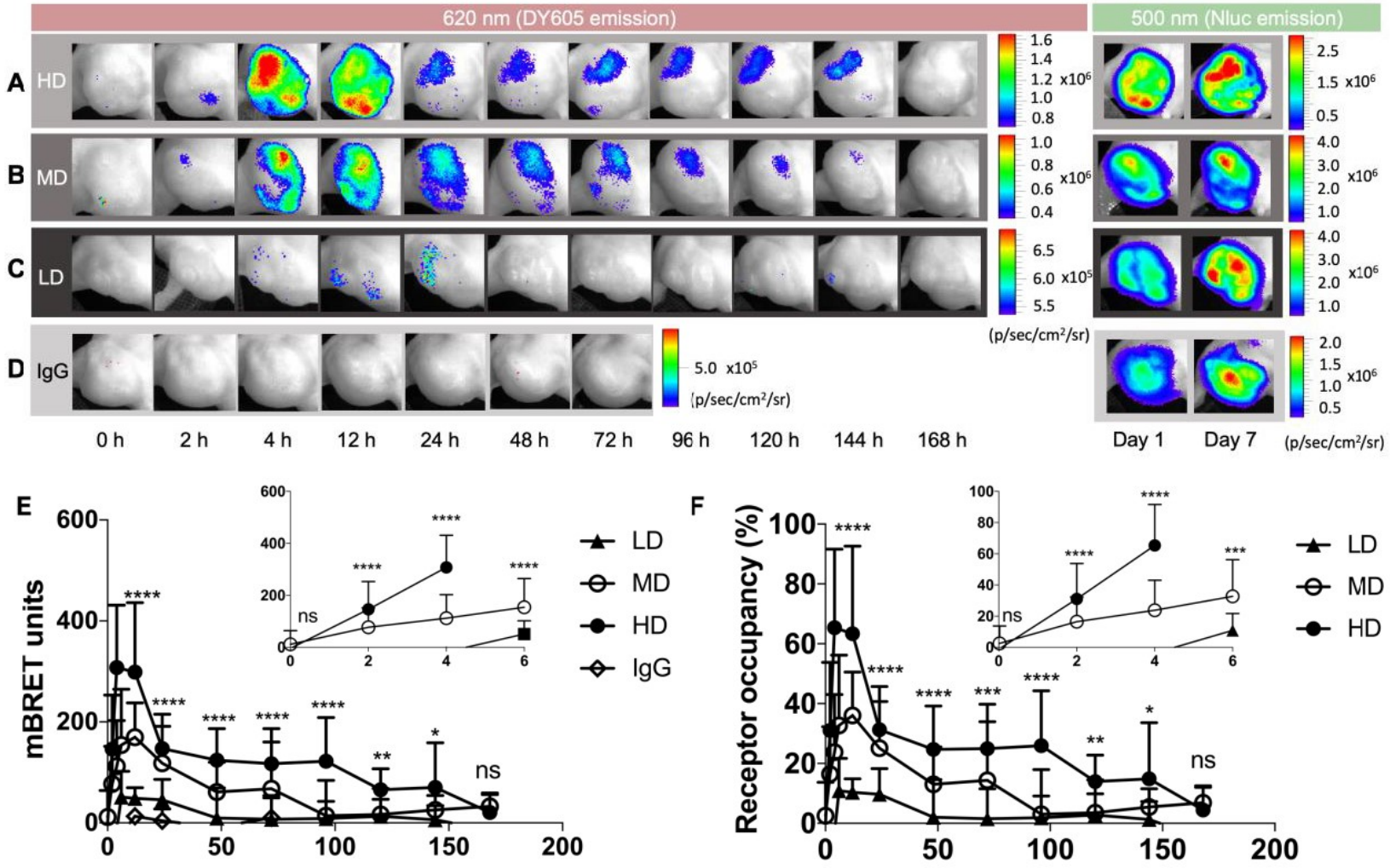
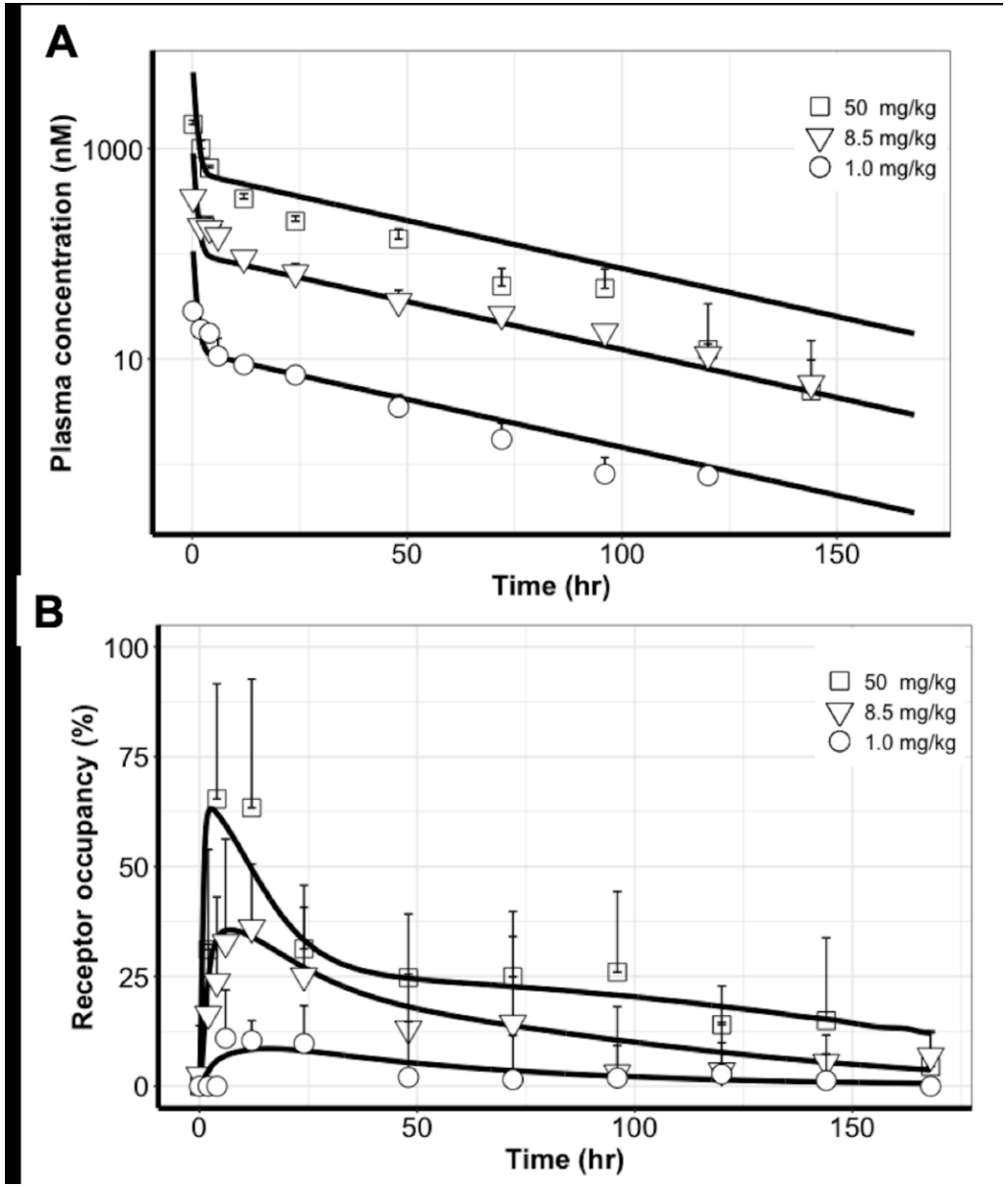
*Tang Y , **iScience***. 2019

Continuously monitored antibody-antigen interaction



*Tang Y , **iScience**. 2019*

We observed:

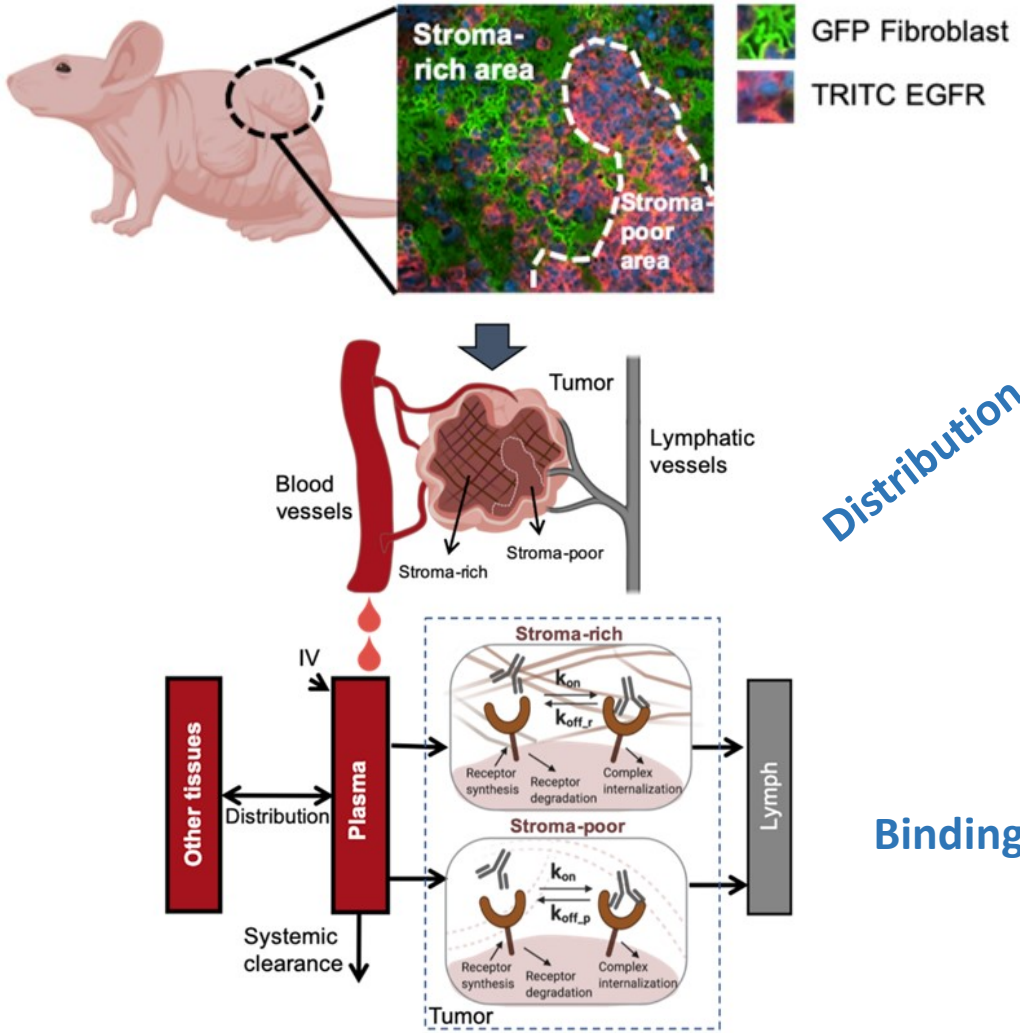


- **Incomplete receptor occupancy in solid tumors, even at supre-therapeutic doses.**

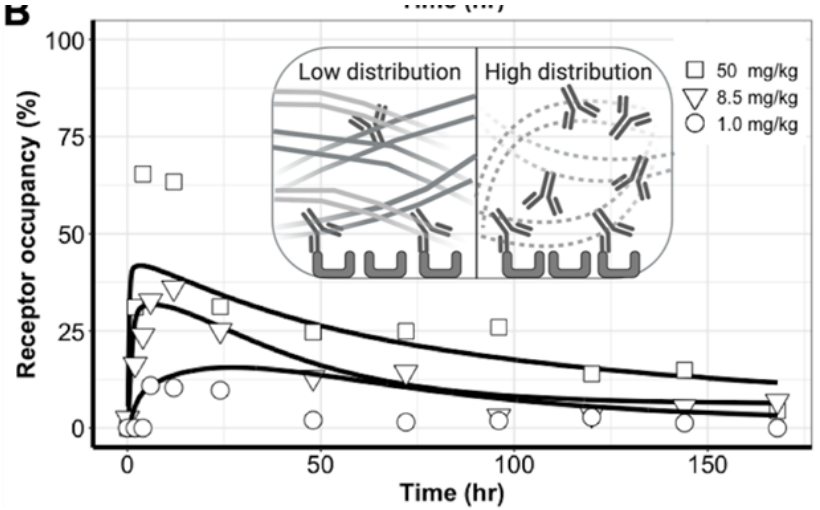
- **A kinetic disassociation exists between plasma antibody and bound targets in tumors.**

Tang Y, ***Sci Rep.*** 2020
Tang Y, ***iScience***. 2019

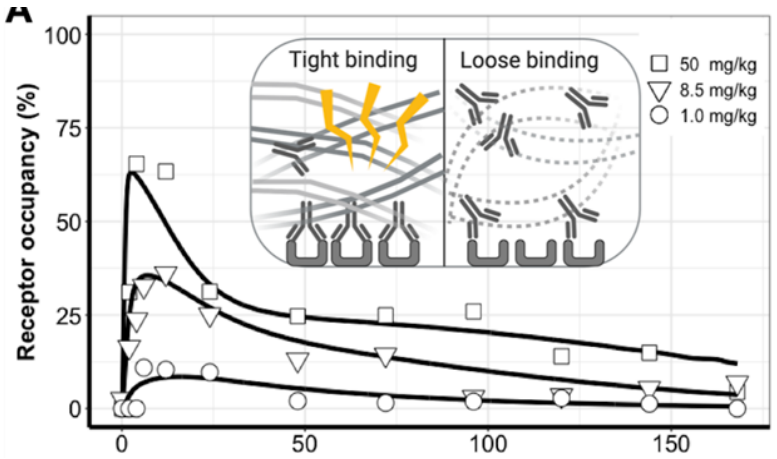
Different Binding Constants between Tumor Areas



Distribution gradient



Binding gradient



*Tang Y, **Sci Rep.** 2020.*

“Slower-but-Tighter" Binding in Stroma-rich Area

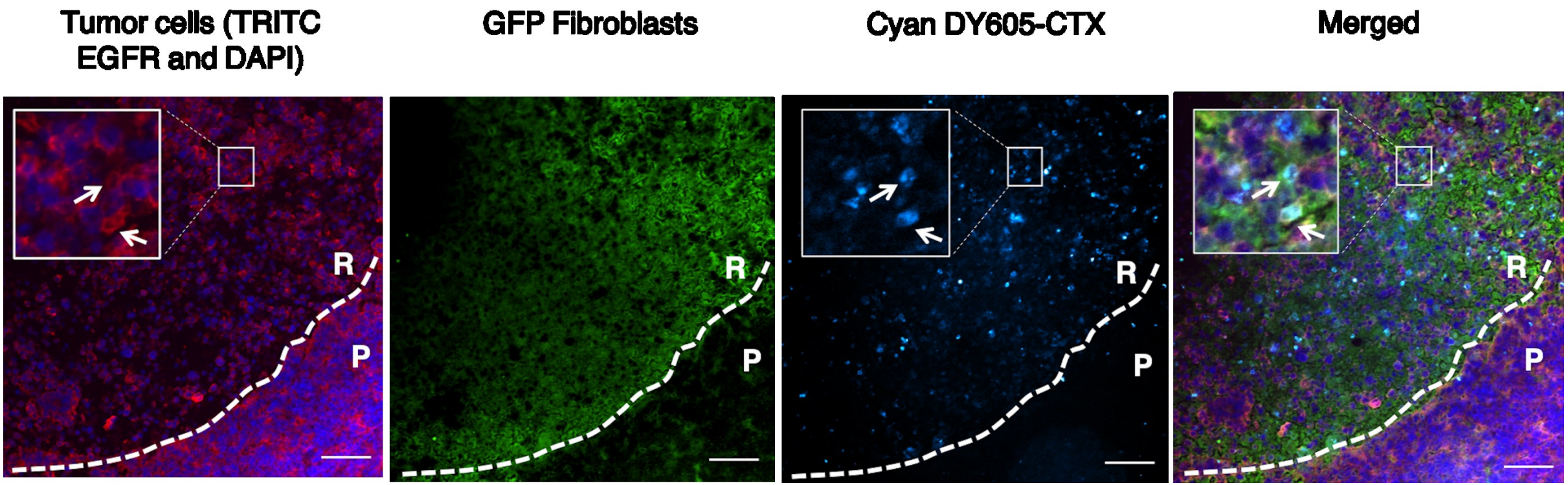
Parameter estimations

Parameter	Unit	Definition	Estimation (CV%)
k_{on}	$nM^{-1} \cdot h^{-1}$	Cetuximab-EGFR apparent association rate	0.030 (53%)
k_{off_p}	h^{-1}	Cetuximab-EGFR apparent dissociation rate in stroma-poor regions	**0.61** (55%)
k_{off_r}	h^{-1}	Cetuximab-EGFR apparent dissociation rate in stroma-rich regions	**0.0017** (54%)

~ 300-time difference

*Tang Y, **Sci Rep.*** 2020.

Antibody Persisted Longer in the Stroma-Rich Area



Tumor samples were collected at the end of the imaging study (8 days post-dosing) when the blood antibody has eliminated (close to LOQ).

Tang Y, ***Sci Rep.*** 2020.

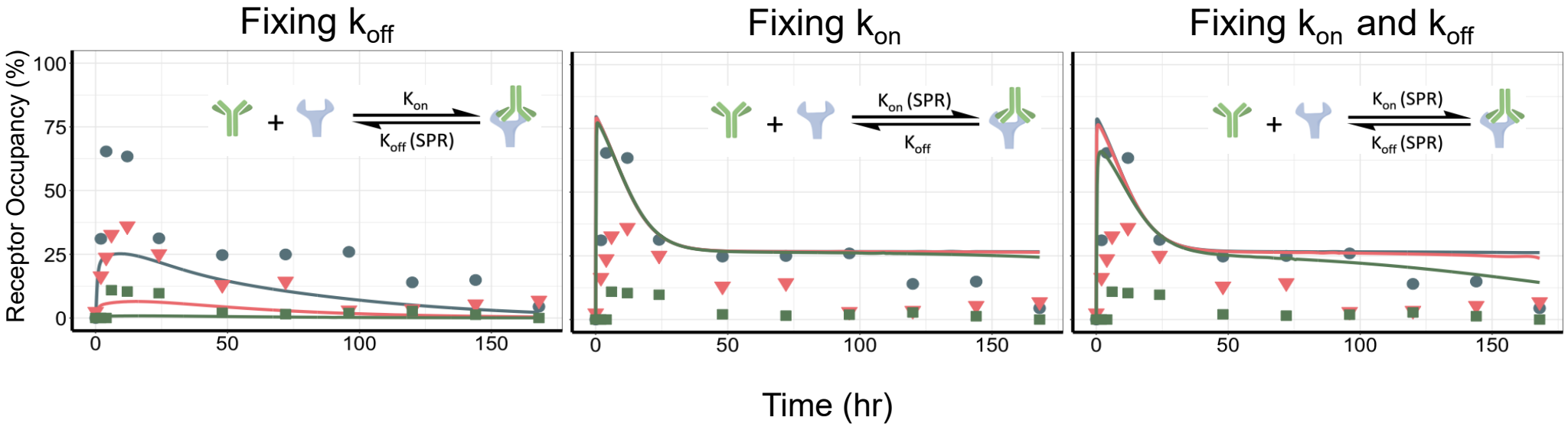
Different Binding Constants between Close and Open Systems

Parameter estimations

Parameter	Unit	Definition	In vitro	Estimation (CV%)
k_{on}	$nM^{-1} \cdot h^{-1}$	Cetuximab-EGFR apparent association rate	2.56	0.030 (53%)
k_{off_p}	h^{-1}	Cetuximab-EGFR apparent dissociation rate in stroma-poor regions	2.88	0.61 (55%)
k_{off_r}	h^{-1}	Cetuximab-EGFR apparent dissociation rate in stroma-rich regions		0.0017 (54%)

Slower binding

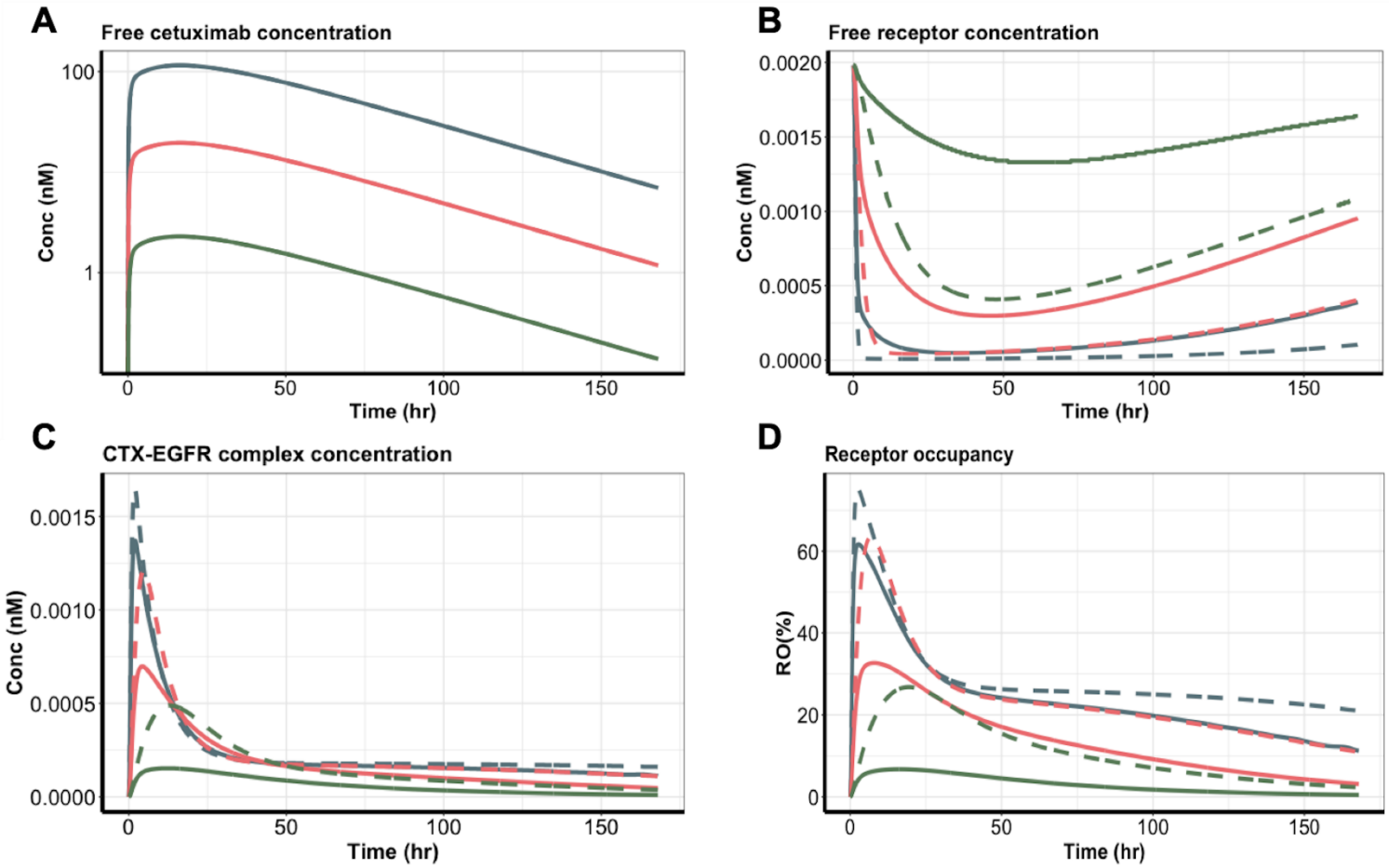
Tighter binding

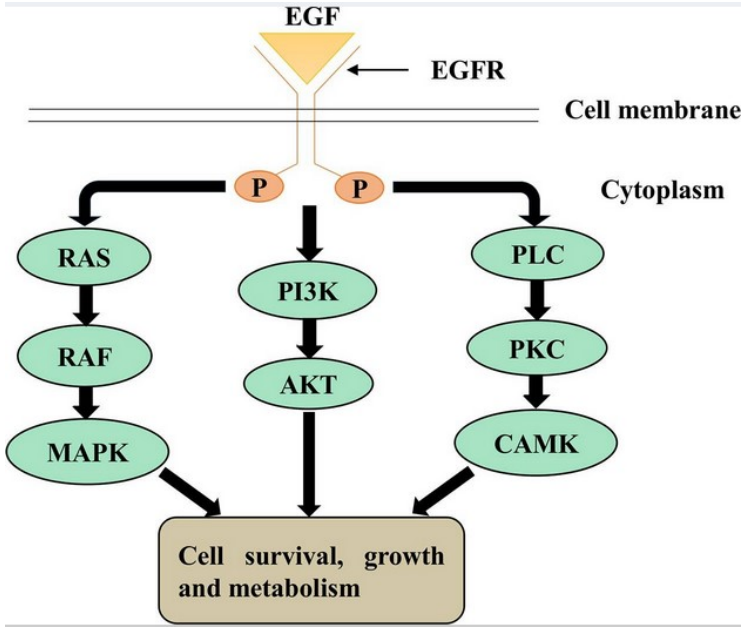


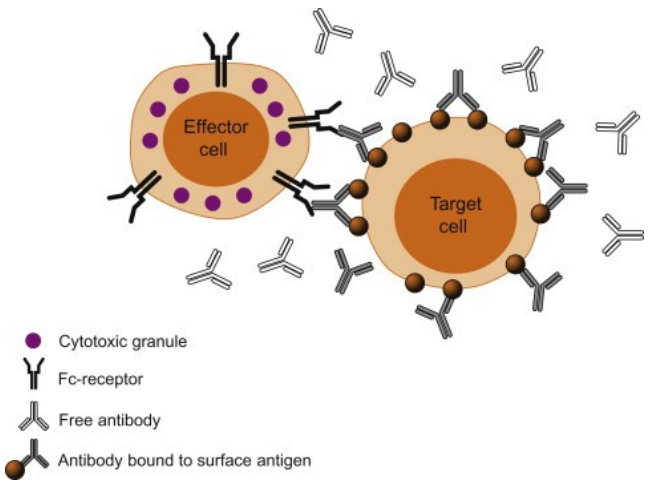
Tang Y Shared Slides

Tang Y, ***Sci Rep.*** 2020.

Antibody-target complex in tumors

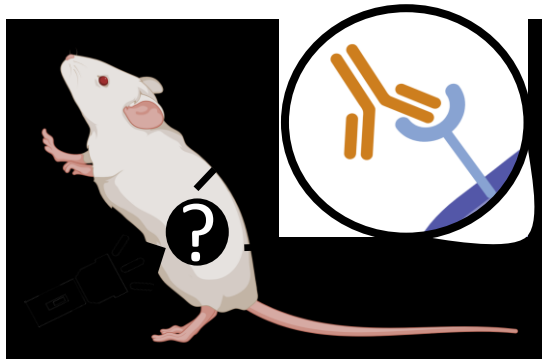






Tang Y, ***Sci Rep.*** 2020.

Summary (Part II)



1. Antibody-target (cetuximab-EGFR) interaction in living tumors was visualized continuously using an BRET imaging method.

2. Cetuximab bound to EGFR to a slower-and-tighter degree in living tumors compared to in the in vitro conditions.

3. Cetuximab persisted longer in the stroma-rich regions than in the stroma-poor regions.

Tang Y, ***Sci Rep.*** 2020.

Limitations and Future Directions

Limitations	Future Directions
Artificial HEK293 xenograft, not equivalent to clinical tumors.	The advanced BRET system can be applied for assessing antibody-target interactions in various tumor types at different locations.
The stromal and cellular molecular mechanisms remain hard to tackle	Other tumor-associated components' effects on antibody-target interactions will be investigated in future studies.
Not yet clinically translational	The spatial receptor occupancy data will be aligned with patient samples (IHC, lesion-specific response)

Tang Y, ***Sci Rep.*** 2020.

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❑ Collaborators
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